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The Effect of Substrate and Environmental Gradients on Attached Diatom Distribution

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Proposal Date
October 1999

Final Report Deadline
May 2000

The effect of substrate and environmental gradients on attached diatom distribution

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Abstract

Sample were taken to determine diatom species distribution and diversity on the Big Island of Hawai'i as related to variable intertidal. Little is known about intertidal diatoms in Hawai'i as very few studies have been conducted on coastal Hawaiian diatoms. Samples were taken from three different locations from three different substrates - rock, seaweed, and the top sediment layer. At each location temperature, salinity, and sample depth were taken. Samples were then cleaned by an acid digestion, mounted, and examined by Nomarski and SEM analysis. Diatoms were identified and enumerated. The relative abundance was calculated for the top ten species overall, the top five species of each sample, and also the top five species from each location. Diversity was calculated using the Shannon Wiener diversity indices. Using anova, regression analysis, and multivariate analyses, it was concluded that diatom distribution and diversity on the Big Island appears to be influenced by seasonal variations. In addition, five of the top ten species showed a preference for different environmental gradients, substrates, and locations.

Background

Diatoms are unicellular plants belonging to the plant class *Bacillariophyceae* of the division or phylum *Chrysophyta*. Diatoms are either solitary or free, attached to a substratum or joined to each other in chains of varying length. They are distributed throughout the world in aquatic, semi-aquatic, and moist habitats. They are found in the sea, estuaries freshwater lakes, ponds, streams, and ditches. Though individual diatom cells are microscopic, masses of diatoms can often be seen on stream bottoms, along surf zones, and during plankton blooms as brownish colored waters or films (Castro & Huber, 1997).

The non-planktonic diatoms found in estuaries and marine coastal waters include taxa that grow attached to other plants (epiphyton), particularly marine angiosperms and macroalgae; on relatively large rocks (epilithon), and on sediments (edaphic). Diatoms associated with sediments are sometimes subdivided further into those taxa that grow

attached to sand grains (epipsammic) and taxa, usually motile forms, that live on silty sediment but are not firmly attached to particles (epipelic).

Epipelic diatoms are usually larger motile forms in contrast to epipsammic diatoms. Intertidal studies as reviewed by Round (1971) suggest that epipelic flora may be restricted to a single species in a particular location, whereas diversity on silty sediments can be very high. Continuous gradients associated with salinity, temperature, and depth also affect distribution (Burckle, 1989).

There appears to be a widespread association of minute epipsammic species and it is speculated that this is a very distinct flora, with some species perhaps restricted to this habitat. The epipsammic individuals belong to non-motile taxa of the *Araphidineae* (*Fragilariales*) or *Monoraphidineae* (*Achnanthales*) and are either attached by a raphe-bearing valve or by the girdle side. Species typically associated with sand may include members of these genera: *Rhaphoneis, Plagiogramma, Dimerogramma, Achnanthes, Cocconeis, and Amphora* (Amspoker, 1973; Burckle, 1989), while epipsammic diatoms exhibit little or no movement, the epipelic forms are usually larger motile diatoms with a well-developed raphe system. It also appears certain from laboratory work and field studies that light rather than tidal cycle is the most important factor controlling the vertical movements of epipelic diatoms (McIntire, 1974).

A distinct epiphytic diatom flora may occur on macroalgae of the intertidal zone. Most individuals are clearly attached by means of mucilage pads or stalks, for example, species of *Cocconeis, Achnanthes, Lismophora, and Synedra.* Many species found in the intertidal zone also extend into the subtidal, but systematic studies are needed to sort out distribution patterns. The relationship between epiphytic associations and such factors as temperature, salinity, depth, and tidal cycle have been investigated. There has been found a preference of different species for different types of thalli. Some species however, show no dependence on the substrates (McIntire, 1974). Host specificity has been a topic of some interest as well. While it is tempting to accept the hypothesis that at least some epiphytic diatoms have co-evolved with specific host plants, there is no convincing quantitative evidence that there are any obligate epiphytic diatoms or that a particular epiphytic taxon can survive only when associated with a particular species of host plant (McIntire, 1974). Although host-epiphyte specificity is questionable, there is no doubt

that many diatoms occur in greater frequencies when associated with macroalgae and marine angiosperms than with non-living substrates (Edsbagge, 1966; Aleem 1950; Hopkins, 1964; Round, 1971; Main & McIntire, 1974). Furthermore, many so-called epilithic diatoms are, in fact, growing epiphytically on other diatoms that are attached to rocks (McIntire, 1974).

Epilithic diatom assemblages similar in species composition have spanned across the globe. There have been suggestions that there are some reoccurring associations in different locations around the world spanning from Swanage, Dorset, UK to Coos Bay, Oregon. Suggestions have been made as well concerning a vertical zonation that was subject to some seasonal modification by climatic conditions. Some of the abundant species are *Achnanthes brevipes, Amphipleura rutilans, Fragilaria striatula, Navicula grevillei, N. ramosissima, Synedra tabulata,* and species of the genus *Licmophora.*

The ecological properties of diatom assemblages can be examined from essentially two points of view: (1) their distributional patterns and community structure and by (2) comparing the distributional patterns with environmental gradients within in the community (e.g. seasonal, salinity, temperature, depth). This study focused on the distribution and diversity of diatom species along environmental gradients including salinity, water temperature, depth, and seasonal variations. This study will address some ecological aspects of marine diatom assemblages with emphasis on the structure of epilithic, epiphytic, epipelic, and epipsammic intertidal assemblages and the general distribution patterns in these assemblages along chemical and physical environmental gradients (McIntire, 1977). Friedrich Hustedt conducted one of the first studies on Hawaiian diatoms in 1942. He looked primarily at diatoms in general, cataloging and describing species of diatoms. In 1976, Eleanor Saboski conducted a study of Hawaiian diatoms. She looked at the physiological ecology of marine diatoms along a beach gradient. Floristic studies conducted on diatoms in Hawai'i have been few and sparse, apparently limited to Hustedt (1942) and Saboski (1976).

My specific research questions are:

1. Are different species appearing at each location?
2. What species are prominent in Hawaiian waters?

3. Do different species have a substrate/water preference?
4. Are species equally distributed across the substrates and also across the environmental gradients?
5. How does my species list compare with that of Hustedt (1942) and Saboski (1976)?

Hypotheses

My hyptheses are:

H_{o1}: Distribution of species will differ between sampling locations

H_{o2}: Species will have a preference for a certain water temperature, salinity, depth, substrate, and season

Materials & Methods

Sampling Locations - Water and species distribution analysis were collected at three sites – Reed's Bay, Richardson's, and Mahaiula (Fig. 1). The sites were chosen because they have all substrate types (rocks, loose sediments, and seaweeds) and variable environmental gradients (salinity, temperature, and depth). Diatom samples were collected once during the fall and winter. The fall sampling dates were October 24, 1999 and October 28, 1999. The winter sampling dates were January 15, 2000 and January 19, 2000.

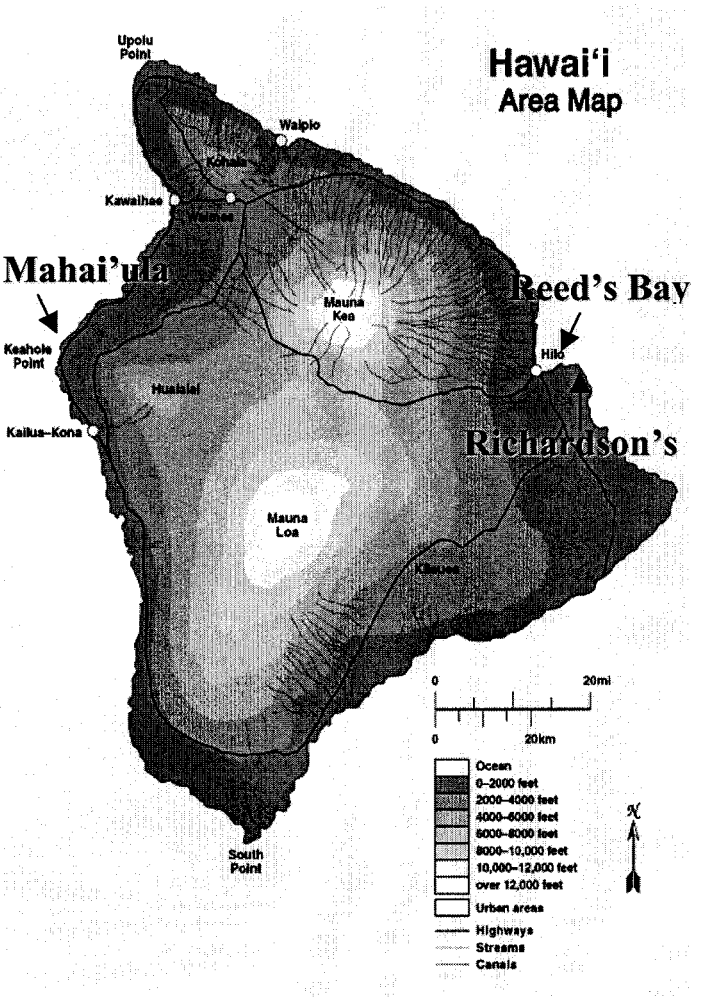

Fig. 1. Sampling locations around the Big Island of Hawai'i shown in bold face print.

Sampling Strategy - Samples were randomly taken at all three sites. A minute amount of diatoms were removed off the substrate using a knife and then placed into a sampling tube. Six samples were collected at each site during the fall and winter. Two samples were collected for each substrate type at each site. In total, there were 36 samples, eighteen of which were replicate samples.

Water Quality Analysis - At each site, temperature and salinity were recorded using a refractometer and thermometer. Depths were also recorded at each sample collection.

Standard Procedure for Diatom Analysis - After the samples were collected, they were cleaned using a nitric acid digestion in order to clean and concentrate diatom valves to facilitate diatom identification and enumeration. The general cleansing procedure is adopted from Parsons (1996):

a. Samples were transferred samples from sample container to polypropylene centrifuge tubes and then centrifuged for 7-10 minutes
b. Using a disposable pipette, the supernatant was removed, distilled water was added to the sample, centrifuged, and followed by supernatant removal (the rinse step)
c. Hydrochloric acid was added a drop or two at a time to completely remove the calcium carbonate material in the sample and the rinse step was repeated
d. After the HCl digestion, the samples were washed and rinsed six times with distilled water and centrifuged for 7-10 minutes on top speed
e. Approximately 3 ml of concentrated nitric acid was added to each sample which were then subjected to a boiling water bath for twenty minutes to remove organic matter and separate and clean diatom frustules
f. After the acid digestion, the samples were washed and rinsed six times with distilled water and centrifuged 7-10 minutes at top speed

Slide Preparation - In order to observe my samples using the Zeiss universal microscope utilizing Nomarski illumination, the clean frustules were placed onto microscope slides. The general procedure is as follows from Parsons, 1996:

a. Each sample was brought up to a volume of 12 ml after which 0.5 ml of subsample was extracted from the center of the tube, using a plastic disposable pipette.
b. The subsamples was agitated, and two drops were placed on a #1 25mm square glass coverslip, and dried on a hotplate at medium heat.
c. The dried coverslip was then permanently mounted onto a microscope slide with two drops of Naphrax® mounting media and placed back on the hotplate to bubble out.
d. Slides were labeled and numbered accordingly

Scanning Electron Microscope Stub Preparation - In order to capture the fine details of each diatom species using the Scanning Electron Microscope, the samples were placed onto SEM stubs using the general procedure as follows from Parsons, 1996:

a. Using an electric pump and a handmade filter system provided by Dr. Michael Parsons (Fig. 2; Scholin et al, 1997), two drops of each sample were dropped into the centrifuge sample containers and filtered through with four rinses.
b. After the fourth distilled water rinse, the top of the filter was removed and then the filter paper was removed.
c. The filter paper was then placed onto a SEM stub using double-sided glue tabs.
d. The stubs were numbered underneath and placed into a sample container.

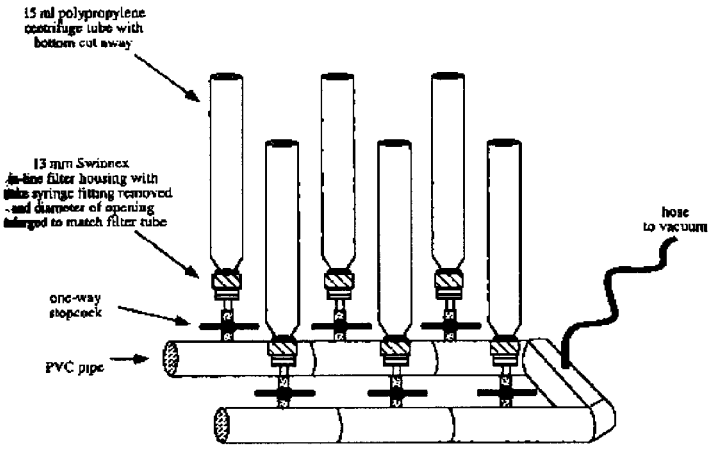

Fig. 2. Schematic showing the configuration of a custom filtration manifold designed to facilitate filter-based whole-cell hybridization.

Sample Archiving Procedure - Samples were placed into containers and went through the following procedure in order to keep samples for later use from Parsons, 1996:

a. Vials were labeled with sample number on bottom of vial.
b. Samples were drawn off until only 4-5 ml of sample was left in the centrifuge tube using a disposable pipette.
c. Samples were transferred to appropriate vials.
d. Two drops of 10% acetic acid were added to the vial.
e. Four – five coverslips were broken in Kim Wipes and to each vial, two small fragments of broken coverslip were added.

Diatom Counts

Nomarski Microscope - Diatom counts were conducted on a Zeiss universal microscope utilizing Nomarski illumination. At least five fields were viewed and fifty valves were counted from each sample in order to determine relative abundance. Diatoms were drawn to as much detail as possible in order to identify the different species in each sample. Diatoms were identified to genus and species level (where possible) with the help from Dr. Michael Parsons of the University of Hawai'i – Hilo and the use of several diatom keys [Cleve et al, 1965; Hustedt, 1962; Hustedt, 1942; Jensen et al, 1985; Krammer et al, 1997, and Simonsen 1987]. In total, there were 151 different

species between the eighteen viewed samples. Due to a lack of time, replicate samples were not viewed.

Scanning Electron Microscope - The Scanning Electron Microscope (Model ISI WV-6, Lab 6 filament, 220 V, 10-15kV) was used to focus on the fine details, to show diversity in both shape and appearance, and to further taxonomically catalog the top ten species.

Data Analysis

Relative Abundance - The number of occurrences of each species on each sample was divided by total number of species counted (50) to determine the relative abundance. The relative abundance data were then used to produce rankings which assembled the top ten species overall, and also the top five species from each site and sample. These ranked data were used for distribution and comparison purposes.

Diversity - Diversity was calculated using the Shannon Wiener Diversity Indices. This order was characterized by the relative abundance to specify the degree of diversity.

$$H' = - \Sigma (p_I) (\log_2 p_I)$$

Diversity was used to investigate distribution among species and also to observe any preferences that may occur in conjunction with the different environmental gradients.

Anova and Regression Analysis - Using my diversity calculations for each slide, I observed distribution across the Big Island and also at the distribution in comparison with the environmental gradients. I used one-way analysis of variance to test the equality of population means by one variable, two-way analysis of variance tested the equality of populations by two variables, and regression analyses to test the relationship between a response variable and one or more predictors. Regression analysis was used for both my top ten overall species and the top five species among location.

Multivariate Analysis - The clustering analysis of observations was used to classify observations into groups when the groups are initially not known. This procedure used an agglomerate hierarchical method that begins with all observations being separate, each forming its own cluster. In the first step, the two observations closest together are joined. In the next step, either a third observation joins the first two, or two other observations join together into a different cluster. We determined that Euclidean

distance and Ward linkage produced the best dendogram. After a cluster analysis was performed on the data, the samples were clustered into cluster 1 or cluster 2. The two clusters were then used in a discriminant analysis to classify observations into two or more groups according to the environmental gradients and location data.

Results

Relative Abundance - After calculating the relative abundance, the species were ranked to find the top species overall on the Big Island of Hawai'i (Table 1).

Table 1. The top species on the Big Island of Hawai'i (*Fragilaria lapponica* being the most abundant and *Nitzschia constricta* being the least abundant species)

Top Species Overall*	**Relative Abundance (%)**
Fragilaria lapponica	12.2
Cocconeis pellucida	6.10
Amphora sp. #1	4.60
Melosira sulcata	3.70
Surirella amphioxys	2.56
Fragilaria sp. #8	2.40
Melosira agassizii	2.30
Nitzschia frustulum	2.00
Cocconeis disculus	1.90
Nitzschia constricta	1.80

*These species can be seen in the Scanning Electron Micrographs in Fig. 3 (Plates 1-3).

In comparison with Hustedt's study of 1942, five of the overall top ten species were also found in his study. *Fragilaria lapponica, Melosira sulcata, Melosira agassizii, Nitzschia frustulum,* and also *Nitzschia constricta* were some of the species within in his species list. In comparison with Saboski's work of 1976, only *Nitzschia constricta* of the top ten from this study was present on her species list. However, all three studies were composed of relatively the same genus'. In addition, we did have a few differing genus' as well.

Also using the relative abundance, the top five overall species per location were ranked (Table2).

Table 2. The top five species per location from most abundant to least abundant.

Mahaiula	Richardson's	Reed's Bay
Fragilaria lapponica	*Fragilaria lapponica*	*Cocconeis sp. #4*
Amphora sp. #1	*Fragilaria sp. #8*	*Fragilaria lapponica*
Melosira sp. #3	*Cocconeis sp. #4*	*Navicula sp. #13*
Navicula lyrella	*Amphora sp. #1*	*Navicula fallacia*
Surirella sp. #7	*Surirella subsalsa*	*Navicula lyrella*

Evidently, there are some species that are the same throughout each location, yet there are different species as well. You can observe the comparison between the overall species and the top five species from each location in Fig. 4. As you can see, *Fragilaria* is still a very dominant species with a greater abundance on the Kona Coast at Mahaiula. Judging by the different colors, it appears that the distribution between locations differs with some similar species between the locations, yet there is no correlation between diversity and location statistically.

In addition to the top species overall, the relative abundance was used to rank the top five species per sample and can be seen in Table 2.

Diversity - Diversity was calculated using the Shannon Wiener Diversity Indices (Table 3):

Table 3. Shannon Wiener Diversity indices per sample.

Sample	Shannon Wiener Diversity
1	2.258710248
3	2.239303381
5	2.798516455
7	3.088204800
9	2.543125691
11	2.497238736
13	2.388657799
15	2.753586601
17	2.755494154
19	3.180684975
21	3.202777350
23	2.930406887
25	2.536909810
27	2.828786933
29	2.849716859

31	3.061058994
33	3.111099236
35	3.097285334

Anova and Regression Analysis - Using analysis of variance and regression analysis, we discovered that diversity is significantly different between seasons with an increase in diversity from fall to winter (Fig. 5). Surprisingly, however, diatom species diversity was not significant between different temperatures, salinities, depths, substrates, or locations (Table 4).

Table 4. Regression and Analysis of variance p-values for Diversity in comparison with environmental variables and location.

	One Way Anova p-value	Two Way Anova p-value	Regression p-value
Diversity vs. Seasons	0.005	0.004	0.005
Diversity vs. Location	0.764	0.578	
Diversity vs. Substrate	0.937		
Diversity vs. Temperature	0.300	0.074	0.063
Diversity vs. Depth	0.824		
Diversity vs. Salinity	0.317		
Div. vs. Seas. vs. Loc.		0.080	

Also using the general linear model of anova, I focused on the top ten species. In this comparison, I was looking for a preference from each of my top ten species. I found that two of the top ten species had a preference for at least one of the environmental gradients, substrates, or location. *Fragilaria lapponica* showed a preference for the Kona Coast at Mahai'ula (p-value = 0.004) and *Fragilaria sp. #8* showed a preference for depth at approximately ten feet (p-value = 0.050). However, this species only showed up at Richardson's on one sampling occasion which accounts for the significance.

Multivariate Analysis - Using a cluster analysis of the top five species per sample, samples were grouped into Cluster 1 and Cluster 2 by using a tree formation (Fig. 6). After discovering Cluster 1 and Cluster 2, the samples were then run through a discriminant analysis. The data concluded once again that diatom assemblages on the Big Island of Hawai'i vary by season (Table 5).

Table 5. Descriminant analysis comparisons for cluster 1 and cluster 2.

Linear Discriminant Function for Group		
Variable	Cluster 1	Cluster 2
Constant	-1902.5	-2058.9
Slide	10.1	11.9
Season	10.4	17.7
Substrate	13.1	10.1
Salinity	-2.3	-1.4
Temperature	141.5	145.7
Depth	1.4	1.3

Discussion

From this study, I found that the key factor in Hawaiian diatom diversity and distribution is based around seasons. In addition, certain species displayed a significant correlation with different environmental gradients, substrates, and location. This study has isolated several of the more widely changing variables in the intertidal zone for testing diatoms that dwell there and has shown that, for the most part, these diatoms are capable of adjusting to environmental changes.

It is interesting to note that Saboski's study in 1976 on the physiological ecology of Hawaiian, marine, psammolittoral diatoms found that salinity had little effect on species, yet temperature had a high correlation coefficient (Saboski, 1976). The results of this study showed little effect of salinity or temperature on species. Temperature may be a key factor in diatom distribution, however the p-value for temperature within this study was found to be just greater than 0.05. Furthermore, during the sampling days during the fall, all three sites were very calm with little wave action and sunny. During the sampling days in the winter, all three sites were receiving high amounts of rainfall and high wave action. This pattern of coincidence is usually not the case. The Kona coast tends to receive more sunlight and less rain than the Hamakua coast. On a typical day on the Big Island of Hawaii, the Kona coast is sunny while the Hamakua coast is showered with heavy rainfall.

The diatoms at all three beaches showed no significance in location and were very similar in species composition. It is possible, then, that the three beaches, in spite of differences, either contain microenvironments which are habitable for most of the residents (benthic diatom species) or contain species of diatoms which can migrate from relatively less to more favorable areas in the intertidal zone. Because the microenvironment of the intertidal zone has never been completely analyzed in Hawai'i, the full extent of all the variables involved have yet to be realized, especially when working with diatoms.

The marine intertidal zone is an environment with parameters that vary both daily and seasonally. The diatoms that inhabit the interstices of the benthic community are subjected to wide fluctuations in light, temperature, salinity, and nutrients (Saboski, 1976). Hawaiian intertidal diatoms are probably subject to wide fluctuations in salinity,

but salinity may not have a strong effect on diatoms, as many diatom species have been reported in both fresh and saltwater (Hustedt, 1927). Because many epipelic diatom species are cosmopolitan in their distribution and are found in the fluctuating environment of estuaries, it has been considered that diatoms along this zone tolerate a broad range of environmental conditions (Admiraal, 1984; Underwood, 1998). Seasonal variations of benthic diatoms have been found throughout the world. However, it is difficult to understand this concept in an area with little to no seasonal changes.

Another factor to take into consideration when focusing on benthic diatoms is that relying on traditional acid cleaning preparations as a sampling strategy for analysis does not permit the distinction between living and dead material (Oppenheim, 1987). The percentage of dead cells on the sediment surface can be high and can increase species diversity when included in the calculation of diversity indexes (Wilson and Holmes, 1981). This could be another factor that is displaying my results as insignificant.

Some other factors that I would include to enhance this study would be nutrients, precipitation, grazing, and also dessication and light exposure. Due to the great amounts of rainfall during the winter months, it would be interesting to look at precipitation data and also nutrient data as a focus of distribution and diversity.

Acknowledgements

I sincerely thank those who helped me with this project. Thank you to John Coney for providing valuable technical support using the Scanning Electron Microscope and for the never-ending computer support. A special thank to Dr. Michael Parsons for his continuous support, patience, inspiration, and the added push at time to keep me going with this project. Thank you as well to many of the senior thesis students for providing ideas, information, and constructive criticisms throughout this study. Finally, I would like to thank Ian Robbins for being the all mighty partner throughout the years! This paper is part of the senior thesis requirements for the marine science degree at the University of Hawai'i at Hilo.

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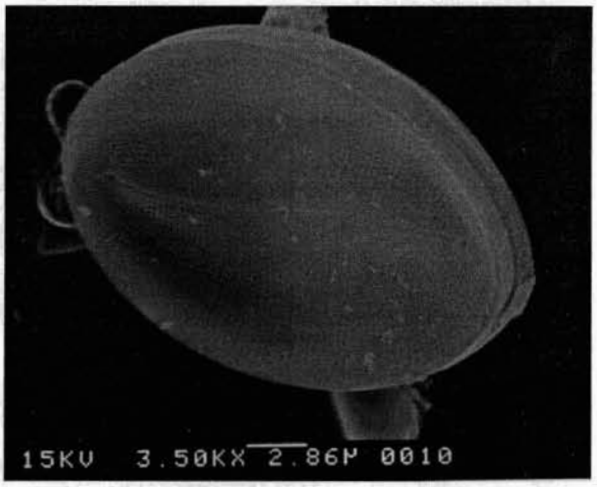

Fig. 3. Plate 1. The top ten species on the Big Island of Hawai'i. At the top is *Fragilaria lapponica* (the most abundant species) and at bottom is *Cocconeis pellucida.*

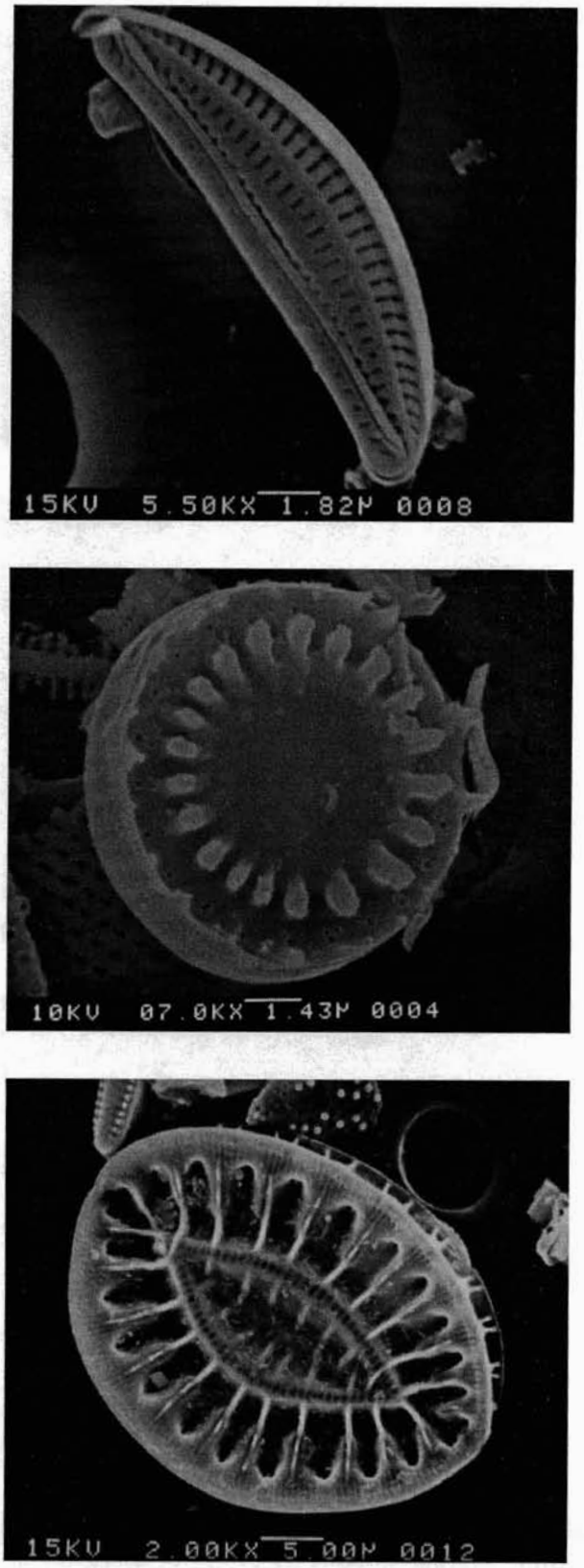

Fig. 3. Plate 2. The top ten species on the Big Island of Hawai'i. At the top is *Amphora sp. #1*, shown middle is *Melosira sulcata*, and at bottom is *Surirella amphioxys.*

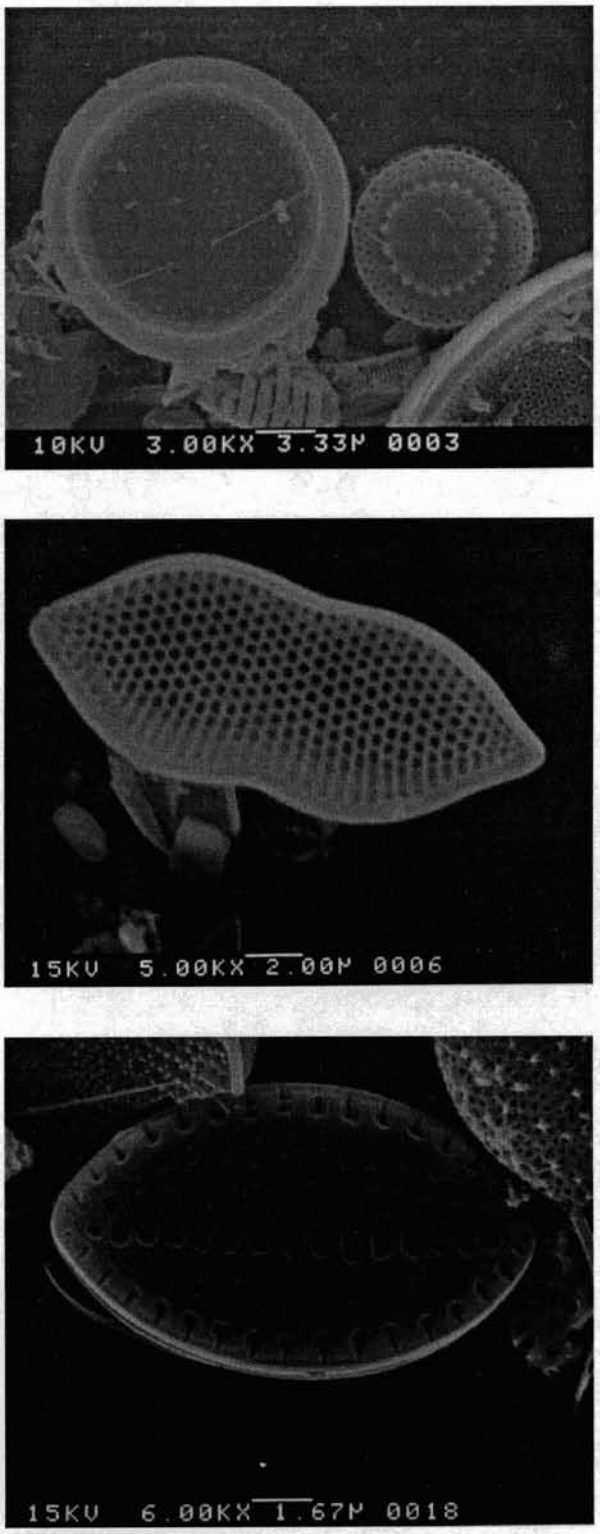

Fig. 3. Plate 3. The top ten species on the Big Island of Hawai'i. At the top is *Melosira agassizii* & *Melosira sulcata*, shown middle is *Nitzschia frustulum,* and at bottom is *Cocconeis disculus.*

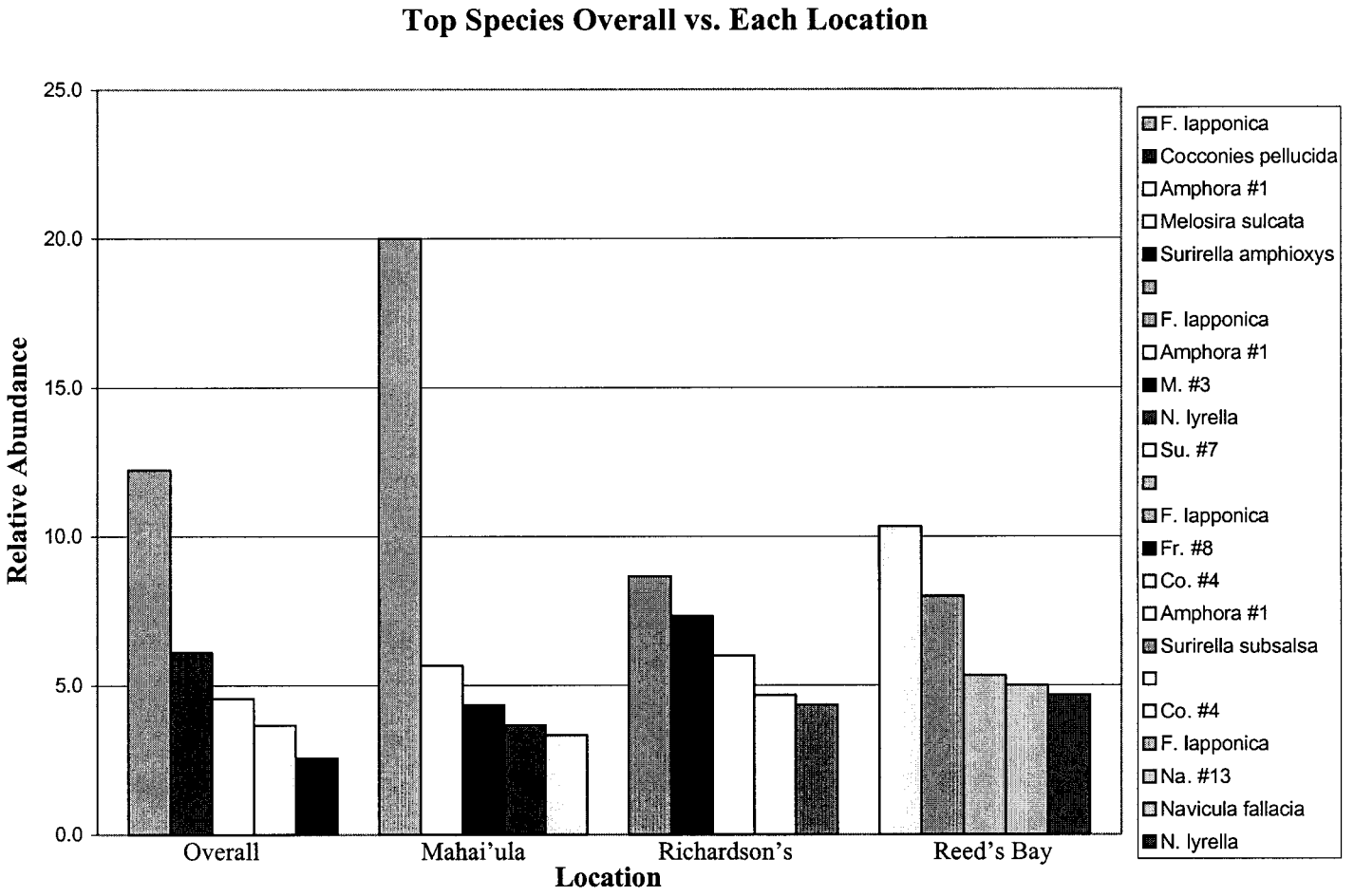

Fig. 4. The comparison between the overall species and the top five species from each location. *Fragilaria lapponica* is significantly dominant with a greater abundance on the Kona Coast at Mahaiula

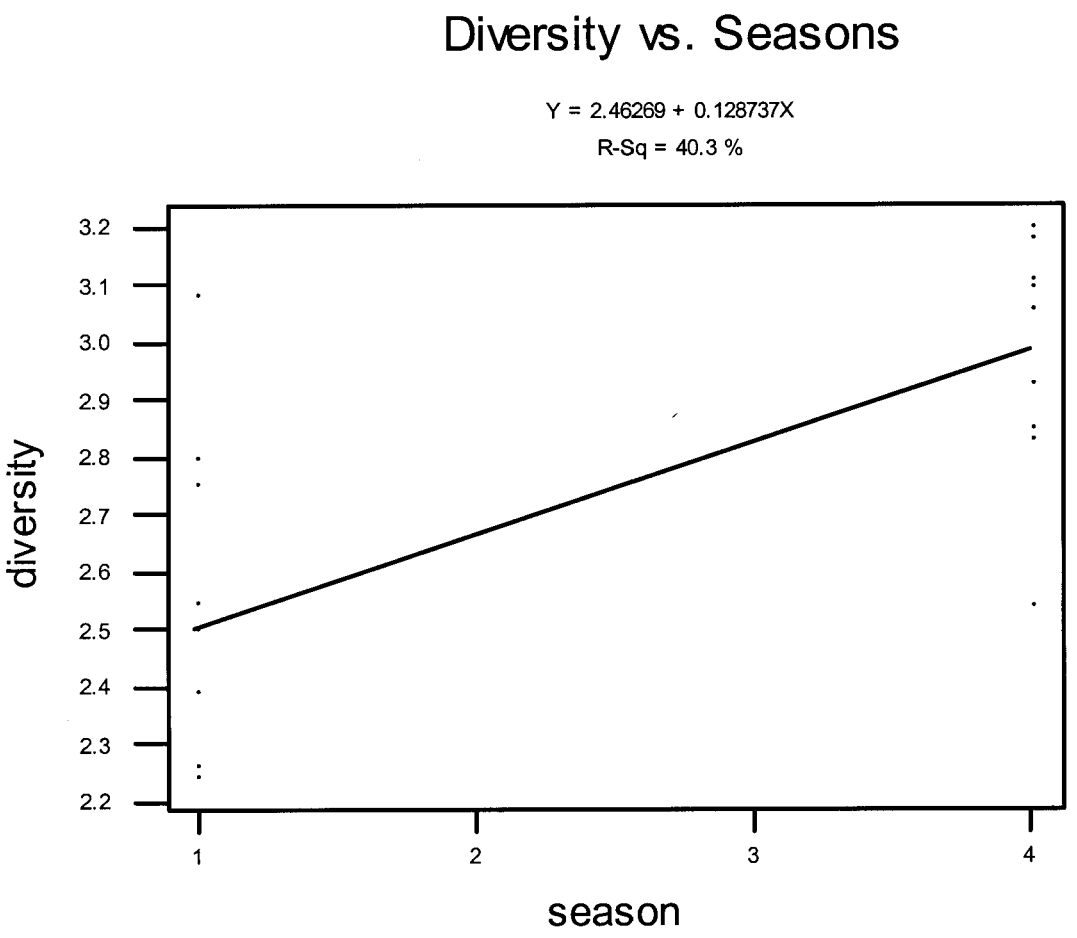

Fig. 5. Regression analysis of diversity versus seasons with a r^2=40.3% and a p-value = 0.005.

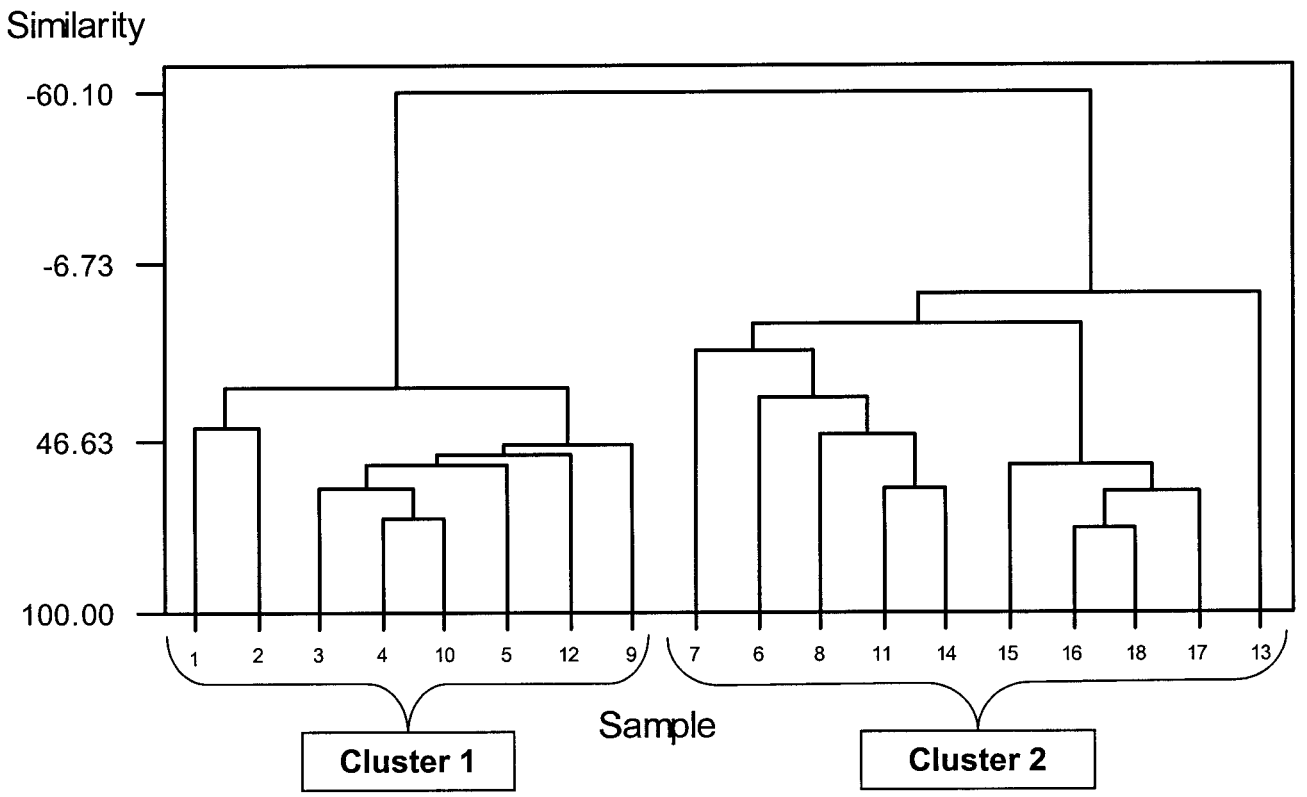

Fig. 6. The clustering analysis of observations was used to classify observations into two separate clusters (shown above) and then was used in a discriminant analysis.